Physiological and biochemical responses of a coralline alga and a sea urchin to climate change: Implications for herbivory

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A B S T R A C T

Direct responses to rising temperatures and ocean acidification are increasingly well known for many single species, yet recent reviews have highlighted the need for climate change research to consider a broader range of species, how stressors may interact, and how stressors may affect species interactions. The latter point is important in the context of plant-herbivore interactions, as increasing evidence shows that increasing seawater temperature and/or acidification can alter algal traits that dictate their susceptibility to herbivores, and subsequently, community and ecosystem properties. To better understand how marine rocky shore environments will be affected by a changing ocean, in the present study we investigated the direct effects of short-term, co-occurring increased temperature and ocean acidification on a coralline alga (*Jania rubens*) and a sea urchin herbivore (*Echinometra lucunter*) and assessed the indirect effects of these factors on the algal-herbivore interaction. A 21-day mesocosm experiment was conducted with both algae and sea urchins exposed to ambient (24 °C, Low CO_2), high-temperature (28 °C, Low CO_2), acidified (24 °C, High CO_2), or high-temperature plus acidified (28 °C, High CO_2) conditions. Algal photosynthesis, respiration, and phenolic content were unaffected by increased temperature and CO_2, but calcium carbonate content was reduced under high CO_2 treatments in both temperatures, while total sugar content of the algae was reduced under acidified, lower temperature conditions. Metabolic rates of the sea urchin were elevated in the lower temperature, high CO_2 treatment, and feeding assays showed that consumption rates also increased in this treatment. Despite some changes to algal chemical composition, it appears that at least under short-term exposure to climate change conditions, direct effects on herbivore metabolism dictated herbivory rates, while indirect effects caused by changes in algal palatability seemed to be of minor importance.

1. Introduction

Current changes in temperature and chemistry of the world's oceans related to anthropogenic activities, chiefly the burning of fossil fuels, have been strongly associated with rapid alterations of marine ecosystems at a global scale (Hoegh-Guldberg and Bruno, 2010; Doney et al., 2012). A major consequence of fossil fuel combustion is the release of CO_2 into the atmosphere, resulting in an increase from a historical 280 parts per million (ppm) to the present-day level of roughly 400 ppm CO_2 in the past 150 years (IPCC, 2014). Over the long term, this is predicted to cause the pH of seawater to drop by ∼0.3–0.5 units by 2100, accompanied by an increase in seawater temperature of up to 4.8 °C (Caldeira and Wickett, 2005; IPCC, 2014). Other short-term climate-change related events, such as anomalous high temperature events (i.e., heat waves), have increased in frequency and intensity by 38% along the world's coastlines over the last 30 years (Lima and Wethey, 2012), and a further increase over the next century is predicted (Easterling et al., 2000; Meehl and Tebaldi, 2004). The occurrence of these events can have devastating impacts on marine ecosystems (Wernberg et al., 2013; Frölicher and Laufkötter, 2018). In addition, greater coastal pH variability and human activities can cause regionally or locally enhanced coastal ocean acidification (OA) (Cai et al., 2011; Waldbusser and Salisbury, 2014).

Studies have shown that these changes are likely to have profound

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consequences for the physiology of individual species, where some taxa may benefit and others will suffer negative consequences (e.g., Harvey et al., 2013; Kroeker et al., 2013a). While the direct effects of increasing temperatures or OA on a wide variety of marine organisms is increasingly well known (e.g., Kroeker et al., 2010; Byrne, 2011), fewer studies have examined the interactive effects of these two factors, or their effects on the interaction between species (Wernberg et al., 2011; Harley et al., 2012; Kroeker et al., 2013b). To complicate predictions even further, by affecting species interactions, indirect effects can reverse the direct effects of climate change (Ghedini et al., 2015; Kordas et al., 2017) and greatly confound predictions of impacts based on single abiotic variables (Connell et al., 2011).

Sea urchins and macroalgae have been widely studied with respect to potential impacts of OA and increased temperature, mainly because of their relevant role in shaping rocky shore communities (e.g., Ruitton et al., 2000; Bulleri et al., 2002). A semi-quantitative global analysis of the literature revealed that sea urchins are surprisingly resilient to OA, with direct impacts being mostly negative but sublethal (Dupont et al., 2010, Dupont and Thorndyke, 2013). Current evidence suggests that the impact of OA on adult sea urchin fitness is either negative (i.e., increased mortality, decreased fecundity or fertilization success, or a sublethal decreased growth rate) or neutral (e.g., Shirayama and Thornton, 2005; see Dupont and Thorndyke, 2013; Brown et al., 2014). A well-documented effect of acute exposure to OA conditions in sea urchins is the induction of extracellular acidosis (Spicer et al., 2011; Dupont and Thorndyke, 2012; Spicer and Widdicombe, 2012; Stumpp et al., 2012), which leads to increased energy costs needed for intracoelomic pH buffering (Catarino et al., 2012). On the other hand, OA has also been reported to directly impact feeding rates and thus affect the total amount of energy available for compensating coelomic acidosis and other processes, such as somatic and gonadal growth (Siikavuopio et al., 2007; Stumpp et al., 2012; Kurihara et al., 2013). It appears that the interaction between temperature and OA on sea urchin metabolism is complex; temperature may be the main driver of change, it may moderate the effects of OA, or it may simply show an additive effect with OA (Byrne, 2011; Catarino et al., 2012; Uthicke et al., 2014; Carey et al., 2016).

In the case of calcareous macroalgae, it is generally understood that they will be highly affected by temperature increase and OA (Doney et al., 2009), experiencing a reduction in biomineralization, whereas non-calcareous algae will become more productive (Doney et al., 2009; Harley et al., 2012; Kroeker et al., 2013b; Johnson et al., 2014). In particular, the increased CO_2 concentrations will increase the solubility of calcium carbonate ($CaCO_3$), resulting in lower calcification rates or even net dissolution of $CaCO_3$ in these algae (Campbell et al., 2014; Comeau et al., 2014; Johnson et al., 2014; Kram et al., 2016). Given that $CaCO_3$ content contributes to the structural integrity and herbivore defenses of calcareous algae (Littler et al., 1983; Lewis, 1985; Paul and Hay, 1986; Hay et al., 1994; Schupp and Paul, 1994), these OA-induced shifts in phytochemistry may play a prominent role in the ability to resist mechanical damage and deter grazing. In addition, calcareous algae exhibit potent herbivore feeding deterrents related to the chemical properties of the algal tissue, such as the concentration of secondary metabolites (Hay and Fenical, 1988), which have been shown to respond to temperature and OA. The response of these metabolites (e.g., phenolic compounds) to climate change seems to be species-specific, ranging from an increase in content (Celis-Plá et al., 2015, 2017; Hargrave et al., 2016; Gouvêa et al., 2017) to no response (e.g., Endo et al., 2013, 2015; Campbell et al., 2014; Vizzini et al., 2017) or a decrease in content (e.g., Yildiz and Dere, 2015). Given that herbivory is a key structuring agent in algal community composition (Lubchenco and Gaines, 1981; Hay and Fenical, 1988), especially in rocky intertidal zones (Poore et al., 2012), both qualitative and quantitative distinctions in the production of phytochemical compounds and the effect on herbivore feeding is of primary importance for the broader ecology of marine habitats.

Studies of the effects of acidification and increased temperature on plant-herbivore interactions have given much attention to the role of defenses against herbivory. Some studies state these conditions will cause a strengthened interaction, with increasing algal consumption rates by herbivores by either increased temperature (O'Connor, 2009; Poore et al., 2013), OA (Campbell et al., 2014; Duarte et al., 2016) or a combination of both factors (Johnson and Carpenter, 2012). Such experiments often focused on subjecting only plants or only herbivores to climate change conditions, and subsequently testing interactions in control conditions (i.e., Johnson and Carpenter, 2012; Campbell et al., 2014). However, in recent experiments, when both plants and herbivores were subjected to experimental conditions, it has been revealed that indirect effects caused by biotic interactions may amplify or moderate effects to individual species (i.e., Alsterberg et al., 2013; Brown et al., 2014; Poore et al., 2016; Cardoso et al., 2017; Goldenberg et al., 2017; Manríquez et al., 2017; Schram et al., 2017). In addition, there is also evidence that algal nutritional quality (i.e., nitrogen content, sugar content, etc.) may be altered under higher temperatures and acidification, which may impact feeding rates of sea urchins (Brown et al., 2014; Manríquez et al., 2017) and other marine herbivores, such as amphipods (Duarte et al., 2016).

Here, we examined the influence of short-term OA and elevated temperature conditions on 1) the physiology and phytochemical composition ($CaCO_3$, total sugar, and phenolic content) of a coralline alga (*Jania rubens*), 2) oxygen consumption rates of a sea urchin species (*Echinometra lucunter*), and 3) explore the implications of their responses in the context of the algal-herbivore interaction (algal consumption by sea urchin). This information will help to better understand the role of climate-change related stressors in structuring coastal marine trophic webs, beyond individual species.

2. Materials and methods

2.1. Sampling site and collection

Specimens of *J. rubens* and *E. lucunter* (selecting for organisms of similar sizes) were collected in the rocky intertidal zone in Barra da Lagoa and Armação, Florianópolis, Brazil (27.573767° S, 48.420295° W and 27.749376° S, 48.502852° W) in November (late Austral spring) 2016. The mean annual sea surface temperature (SST) in this region is 22 °C, with spring/summer SST of 24.5 °C (max. 26 °C). The region suffers from anomalous high temperature events (heat waves) that have increased in frequency and intensity during the last 15 years, with maximum SST increases of 3–4 °C (Gouvêa et al., 2017).

Both species, *J. rubens* and *E. lucunter*, occur in high abundance in the intertidal zone in the study region, with reported *E. lucunter* densities of 5 individuals per m^2 (Anderson et al., 2011; Labbé-Bellas, 2013). Previous studies of gut content analysis have shown that *Jania* sp. often appear in the diet of *E. lucunter* (Mendes and Tavares, 2006; Reyes-Luján et al., 2015). In addition, we conducted a preliminary feeding experiment with the most abundant macroalgal species at the collection site (*J. rubens*, *Pterocladiella* sp., *Sargassum* sp., and *Ulva lactuca*) and found that *E. lucunter* readily consumed *J. rubens* and showed a high preference for it compared to the other algal species offered.

Immediately after collection, algae and urchins were placed into coolers and transported to the mesocosm facility at the Universidade Federal de Santa Catarina, where the algae were meticulously cleaned of epibionts and meiofauna before placing them into tanks, separately from the urchins until the experiment began. A three-day acclimatization period for both algae and sea urchins was used to slowly increase temperatures to treatment levels. After this acclimatization period, the mesocosm experiment was initiated.
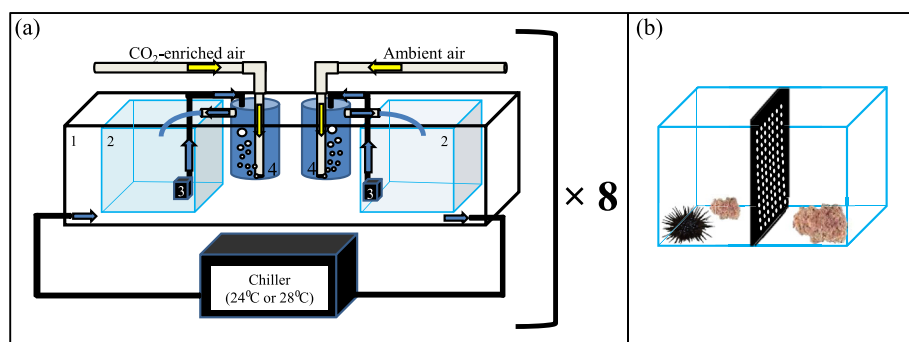
Fig. 1. Schematic representation of the experimental set-up used for the study. (a) An experimental unit consisted of a large tank, acting as a water bath (1), in which two treatment tanks (2) were immersed. Water within treatment tanks was constantly circulated via water pumps (3) into separate mixing tanks (4), where either CO_2-enriched air or ambient air was bubbled to attain the desired CO_2 concentrations. Blue arrows indicate the flow of water through the tanks; yellow arrows indicate the flow of treatment air into the system. A total of eight water bath tanks were used for the experiment, with four set to 24 °C and four set to 28 °C. (b) Close-up of a treatment tank, where sea urchin (+ algae as food) and algae were kept together, separated by a barrier. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.2. Experimental setup

The study was performed in a closed, recirculating mesocosm system under natural light conditions for 21 days (see Fig. 1). The photoperiod during the experiment was 14 h light:10 h dark, with maximum daily irradiances ranging between 750 and 1050 μmol photons $m^{-2} s^{-1}$ (obtained from the Santa Catarina's Agricultural Research and Outreach division-EPAGRI). A total of eight tanks (100 L) were used to maintain the temperature in smaller tanks (38 L), two of which were immersed in each 100-L tank (Fig. 1a), resulting in 16 treatment tanks. Water temperature was controlled using several 300 W aquarium heaters and a chiller (Chiller Radical, Brazil), set to the desired temperature. In each 100-L tank, one of the smaller tanks received ambient CO_2 treatment (Low CO_2) and the other received a "future" CO_2 treatment (High CO_2) (Fig. 1a). The concentration of CO_2 in the water was controlled by an IRGA (Infrared Gas Analyzer LIQUOR 7550) and monitored by an independent IRGA PP SystemEGM (EGM-4 Environmental Gas Monitor for CO_2) (described in Silva et al., 2008). The chosen temperatures were 24 °C (temperature recorded in the field at time of sample collection) and 28 °C. The higher temperature treatment was chosen to simulate a sudden increase in temperature, which was based on a reported maximum temperature spike due to a heat wave in the sampling region (Gouvêa et al., 2017). Air and a CO_2 mix prepared in a separate mixing tank, for ambient (Low CO_2) and the "future" CO_2 treatment (High CO_2), respectively, was injected into seawater within a separate container and pumped into the respective treatment tanks (Fig. 1a), and recirculated at a rate of 60 L h^{-1}. A second pump was placed in each tank to ensure adequate within-tank circulation. A total of 10 L of seawater was replaced in each treatment tank every other day, resulting in a ~12.5% day^{-1} water renovation. Water quality characteristics (temperature, pH, salinity, total alkalinity) were monitored throughout the experiment to calculate carbonate system parameters, using CO2Sys (Table 1). The design resulted in a fully factorial experiment with two temperature levels (24 °C and 28 °C) and two CO_2 levels (Low CO_2 and High CO_2), with n = 4 replications.

Sea urchins and algae were kept together in the treatment tanks, separated by a barrier (Fig. 1b). Upon initiating the experiment, a known quantity of algae was placed with *E. lucunter* for food. Each week, unconsumed algae with *E. lucun*ter was measured to determine feeding rates; then, a known quantity of algae was transferred over the barrier for the subsequent week of feeding. This allowed for algae to be

exposed to the same treatment conditions as *E. lucunter* throughout the experimental period. At the end of the experiment, determinations of photosynthesis and respiration of *J. rubens* and respiration of *E. lucunter* were performed and algal samples were dried and frozen for subsequent $CaCO_3$ and biochemical analysis, respectively.

2.3. Oxygen production and consumption measurements

The algae were incubated with filtered seawater from the respective treatment tanks in 960 ml gas-tight incubation chambers with magnetic stirring to ensure adequate water circulation. Chambers was immersed in a water bath with 50 W aquarium heaters to maintain the same temperature as their respective experimental conditions (24 °C or 28 °C). Initial oxygen concentration of the seawater was measured with a benchtop oximeter (YSI 5000, Ohio, USA) before organisms were placed in the chambers and quickly sealed. *Jania rubens* were incubated for one hour under saturating light conditions (650 μmol photons $m^{-2} s^{-1}$) before oxygen concentrations were re-measured. The same protocol was used for the 20-min dark incubations, which immediately followed the light incubations. For both light and dark incubations, blank chambers with only seawater from the respective treatment tank were incubated to monitor and correct for any background microbial oxygen production/consumption. Oxygen production/consumption was measured as the difference in oxygen concentration from initial and final incubation times, normalized for wet weight of the individual algae. Respiration of *E. lucunter* was measured during dark incubations in the same manner as described above for *J. rubens*. The rates were expressed per sea urchin, after a size correction was applied, using the test diameter as measure of sea urchin size, based on the assumption of a linear relationship between respiration and urchin size (within a limited size range) (see Moore and McPherson, 1965).

2.4. Biochemical analyses of the algae

Biochemical composition of *J. rubens* from the different treatments was evaluated at the end of the experiment. To assess $CaCO_3$ content, samples of *J. rubens* were dried at 60 °C for 24 h, weighed, and afterwards submerged in a 10% hydrochloric acid solution for 30 min. Samples were then gently rinsed with distilled water, dried again at 60 °C for 24 h and re-weighed. The $CaCO_3$ content was calculated from the weight difference before and after decalcification of the tissue and

Table 1
Carbonate system parameters. Values calculated using CO2SYS with salinity, temperature, total alkalinity and pH as input parameters.

| Treatment	Temperature(°C)	Salinity (ppt)	TA (μmol kg^{-1})	pH	DIC (μmol kg^{-1})	HCO_3 (μmol kg^{-1})	CO_3 (μmol kg^{-1})	pCO_2 (ppm)	Ω_{Ca}	Ω_{Ar}
24°C-Low CO_2	23.7 ± 0.1	35.5 ± 0.2	2168 ± 123	8.23 ± 0.01	1860 ± 110	1638 ± 97	213 ± 18	357 ± 25	5.1 ± 0.4	3.4 ± 0.3
24°C-High CO_2	23.7 ± 0.1	35.6 ± 0.1	2602 ± 69	7.75 ± 0.01	2502 ± 68	2360 ± 64	101 ± 3	1378 ± 27	2.4 ± 0.1	1.6 ± 0.1
28°C-Low CO_2	27.4 ± 0.3	36.3 ± 0.2	2227 ± 113	8.28 ± 0.01	1845 ± 109	1659 ± 133	273 ± 6	357 ± 30	6.6 ± 0.1	4.4 ± 0.1
28°C-High CO_2	27.4 ± 0.3	36.1 ± 0.04	2619 ± 49	7.78 ± 0.01	2481 ± 46	2323 ± 43	124 ± 3	1297 ± 23	3.0 ± 0.1	2.0 ± 0.1
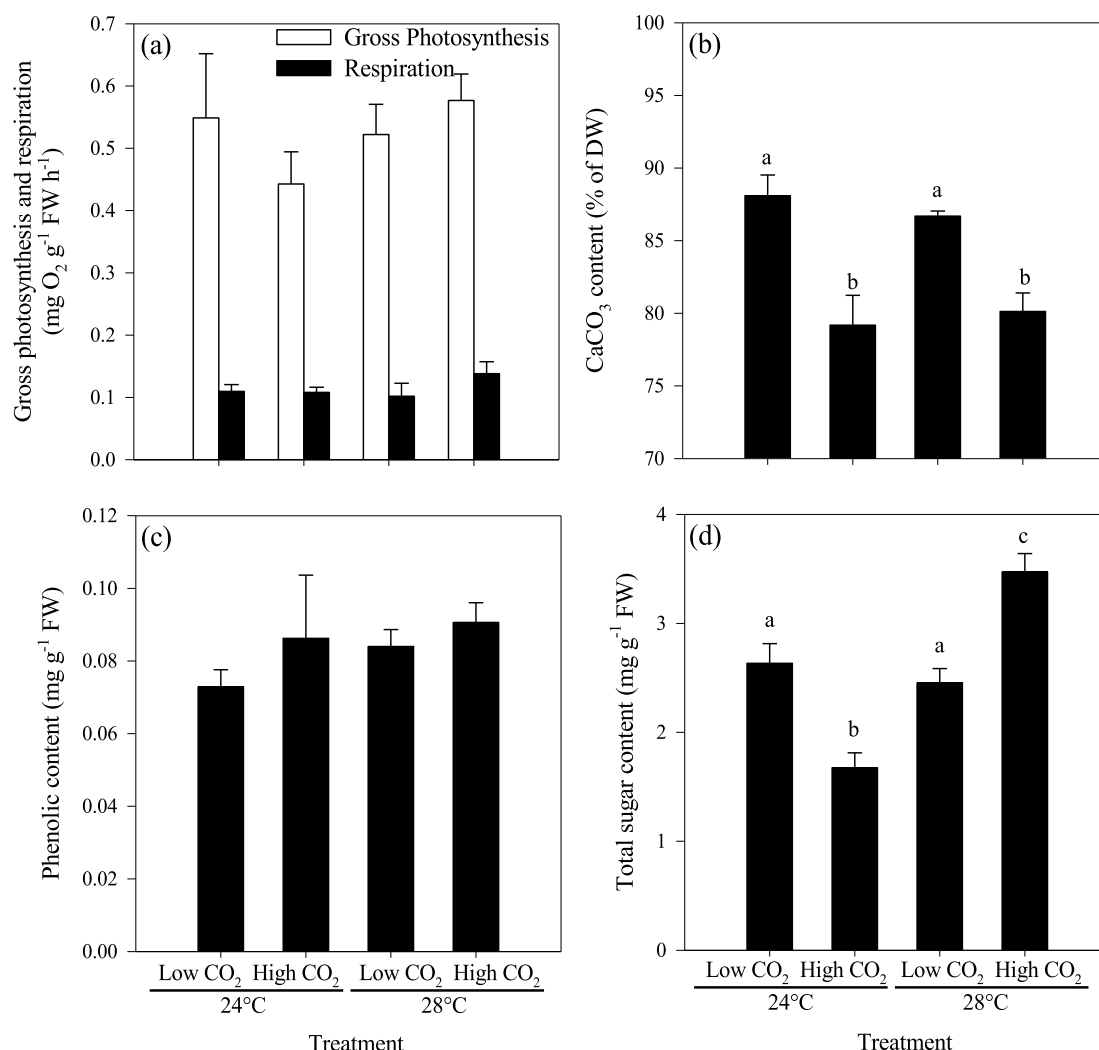
Fig. 2. Response of *J. rubens* to different temperature and CO_2 treatments. (a) Photosynthesis and respiration, (b) $CaCO_3$ content, (c) phenolic content, and (d) total sugar content. Data present mean ± SE (n = 4) and different letters indicate significant statistical differences (ANOVA, $p < 0.05$, Newman Keuls).

expressed as percentage of dry weight.

Extraction of phenolic compounds followed Schiavon et al. (2012) with some modifications. Samples (~300 mg fresh weight) were ground with liquid nitrogen and 8 ml 80% methanol solution was added. After a 1-h dark incubation, samples were centrifuged for 10 min at 4400 rpm 200 μl of the supernatant was pipetted to a separate glass tube; then, 150 μl of folin and 1650 μl of a 2% $NaCO_3$ solution were added. Following a 1-h dark incubation, absorbance of samples was read in a spectrophotometer at 750 nm.

Total sugars were extracted according to Shannon (1968). Algal samples of 50 mg fresh weight were extracted with a 2 ml solution of 12:5:3 methanol:chloroform:water and centrifuged for 10 min at 3000 rpm. The supernatant was removed and 1.5 ml of a 2:3 chloroform:water solution was added before being centrifuged for 10 min at 3000 rpm. This resulted in a two-phase solution, from which the upper phase was extracted and 0.2% anthrone was added, in accordance to Umbreit et al. (1957), and the samples were measured spectrophotometrically (Bel Spectro LGS53, BEL Analytical Equipment Ltd., Brazil). Sugar concentration was calculated using D-glucose as standard.

2.5. Determination of feeding rates

Herbivory rates were measured weekly by placing a known quantity of *J. rubens* with each sea urchin from their respective treatment tanks. Algal samples were weighed prior to being placed with sea urchins, and the remaining quantity of the algae after one week was weighed again. Herbivory was calculated as the difference between initial and final algal biomass, corrected for algal growth during the same period. Growth rates of *J. rubens* were monitored weekly by determining changes in wet weight; however, weekly growth rates were found to be negligible during the experiment. The feeding rates were expressed per sea urchin, after correction as described above for respiration, assuming an inverse relationship of feeding with test size (Lawrence, 1975; Kawamata, 1997).

2.6. Statistical analysis

Statistical analyses were performed using the software Statistica. Interactive and isolated effects of temperature and CO_2 concentrations were evaluated for total alkalinity, algal photosynthesis, respiration, $CaCO_3$ content, phenolics and sugar content, and for sea urchin respiration, using two-way ANOVA. For weekly feeding rates, three-way ANOVA was used to determine isolated and interactive effects between temperature, CO_2 and time. Newman-Keuls Significant Difference post hoc tests were used to identify the statistically different groups. Homogeneity of variance was tested *a priori* using Cochran's test.

Table 2
Summary of two- and three-way ANOVAs, examining the influence of temperature and CO_2 (and time, in case of the feeding rates) on measured parameters.

Species	Model Source	MS	F-value	P-value
Jania rubens	**Gross photosynthesis**			
	CO_2	0.0025	0.178	0.681
	Temperature	0.0106	0.772	0.398
	$CO_2 \times$ Temperature	0.0239	1.74	0.214
	Respiration			
	CO_2	0.0011	1.55	0.239
	Temperature	0.0005	0.637	0.442
	$CO_2 \times$ Temperature	0.0013	1.86	0.200
	Carbonate content			
	CO_2	132.5	7.84	**0.017**
	Temperature	0.1754	0.010	0.920
	$CO_2 \times$ Temperature	0.8025	0.047	0.831
	Phenolic content			
	CO_2	0.0003	1.22	0.298
	Temperature	0.0002	0.73	0.414
	$CO_2 \times$ Temperature	0.00004	0.136	0.721
	Total sugar content			
	CO_2	0.00	0.00	0.985
	Temperature	1.96	27.39	**< 0.001**
	$CO_2 \times$ Temperature	2.93	41.08	**< 0.001**
Echinometra lucunter	**Respiration**			
	CO_2	0.05601	6.386	**0.032**
	Temperature	0.0244	14.678	**0.004**
	$CO_2 \times$ Temperature	0.0507	13.288	**0.005**
	Weekly feeding rates			
	Time	3.69	2.19	0.128
	CO_2	2.84	1.69	0.203
	Temperature	3.84	2.29	0.141
	$CO_2 \times$ Temperature	21.75	12.94	**0.001**
	Time $\times CO_2$	0.8169	0.49	0.619
	Time $\times$ Temperature	0.8410	0.50	0.611
	Time $\times CO_2 \times$ Temperature	0.4433	0.26	0.77

3. Results

3.1. Experimental conditions

Experimental conditions successfully maintained constant temperatures (23.4 ± 0.1- "24°C-treatment"; 27.4 ± 0.3-"28°C-treatment") crossed with ambient ("low-CO_2 treatment") and high CO_2 treatments (Table 1). The average seawater pH was 8.23–8.28 and 7.75–7.78 for the low- and high-CO_2 treatments, respectively. Total

alkalinity (TA) differed significantly between CO_2 treatments (ANOVA, $p < 0.0001$), with higher values in the high-CO_2 treatments (Table 1). The remaining carbonate parameters yielded an average pCO_2 of 357 and 1338 µatm for the low- and high-CO_2 treatments, respectively (Table 1).

3.2. Algal physiological and biochemical response

Gross photosynthesis and respiration rates of *J. rubens* were not significantly affected by temperature, CO_2, or the interaction between these factors (Fig. 2a, Table 2). On the other hand, the $CaCO_3$ content of *J. rubens* decreased slightly but significantly in treatments with increased CO_2 (6–7% lower compared to control treatment), but was not affected by temperature or the interaction between temperature and CO_2 (Fig. 2b, Table 2).

Phenolic concentrations ranged from 0.07 to 0.12 mg g^{-1} FW algae, but were unaffected by the different treatments (Fig. 2c, Table 2). While at 24 °C, sugar content decreased by 36% under high CO_2, but at 28 °C it showed a significant increase under high CO_2, indicating a synergistic effect between the two parameters (Fig. 2d, Table 2).

3.3. Sea urchin physiological and feeding response

Respiration of *E. lucunter* was significantly affected by CO_2, with significantly higher rates in the high CO_2, low temperature treatment; in addition, there was an interactive antagonistic effect of CO_2 and temperature (Fig. 3a, Table 2).

Feeding rates of *E. lucunter* were significantly affected by the interaction between temperature and CO_2 (Table 2). While time did not yield any significant statistical effect, there was a general trend of an increase in feeding over time in all treatments, reaching the highest rates during the third week of the experiment (Fig. 3b). This effect was especially pronounced in the high CO_2 treatment under lower temperature conditions, with a 42% increase in feeding from the first to the third experimental week (Fig. 3b).

4. Discussion

Effects of climate change on species interactions, such as herbivory, will determine ecosystem-level responses under future conditions (Harley et al., 2012). Here, we demonstrate that short-term exposure to climate-change related stressors (increased temperature and OA) induces direct effects on herbivore metabolism and algal nutritional
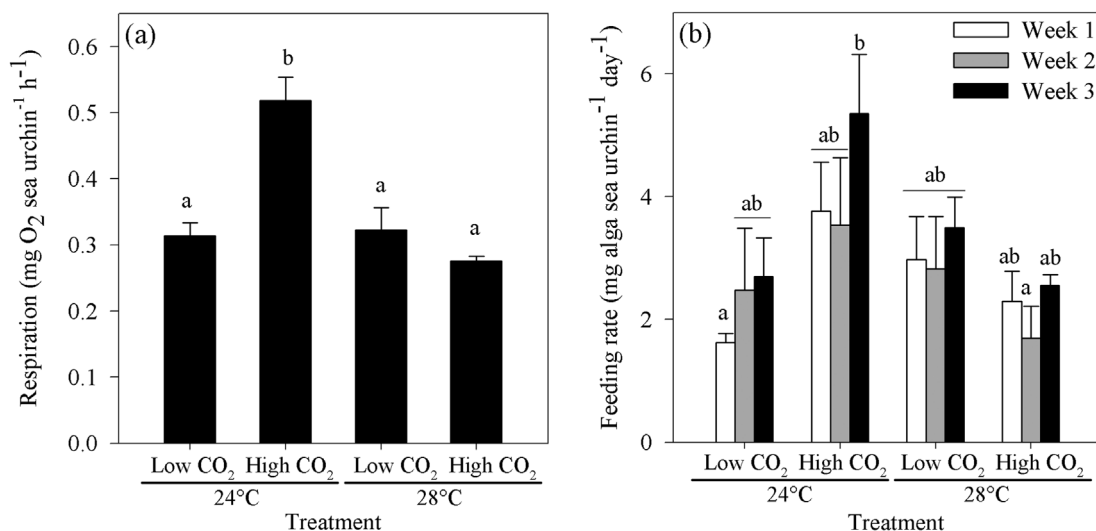

Fig. 3. Sea urchin responses to different temperature and CO_2 treatments. (a) Sea urchin respiration and (b) weekly feeding rates during the experiment. Data present mean ± SE (n = 4) and different letters indicate significant statistical differences (ANOVA, $p < 0.05$, Newman Keuls).

content and defenses, which in turn affects the feeding rates on algae by the herbivore.

In the present study, *E. lucunter* exhibited a significant increase in respiration under high CO_2 in the lower temperature treatment (Fig. 3a). This agrees with the general assumption that increases in respiration under OA conditions reflect increased energetic costs, related to the compensation for the acidosis of the coelomic fluid induced by high seawater CO_2 (Catarino et al., 2012; Dupont and Thorndyke, 2013). Similar findings during short-term exposure have been reported by Suckling et al. (2015), while in other species metabolic rates increased in response to both OA and increased temperature (Catarino et al., 2012; Carr and Bruno, 2013; Carey et al., 2016). In turn, other species' metabolism was unaffected when exposed either to higher temperature (Harianto et al., 2018), OA (Stumpp et al., 2012; Moulin et al., 2014, 2015; Kurihara et al., 2013), or both stressors (Uthicke et al., 2014). A lack of metabolic response to elevated temperature in the current study may be due to the organisms' natural acclimatory ability (e.g., Newell and Branch, 1980; Vargas et al., 2017). The high temperature treatment in this study (28 °C) was 4 °C above the ambient seawater temperature at the time of collection, simulating a sudden increase in temperature experienced during heat wave events (Gouvêa et al., 2017). However, as both *J. rubens* and *E. lucunter* inhabit shallow intertidal coastal areas (< 0.5 m), where they are subjected to variable temperatures, including exposure to air temperatures during low tide, they may be acclimated/adapted to sudden changes in temperature.

In addition to the direct OA effect on *E. lucunter* respiration, the urchin feeding rates on coralline algae, while variable in all treatments, exhibited an increase under these conditions during the third week of the experiment (Fig. 3b). A similar response to high CO_2 conditions was found in the sea urchin *Strongylocentrotus purpuratus* and its consumption of the coralline alga *Lithothrix* sp. (Briggs, 2017), while other studies reported reduced feeding rates under OA conditions (Siikavuopio et al., 2007; Stumpp et al., 2012; Kurihara et al., 2013). In the case of *E. lucunter*, we assume that the increase in algal consumption under OA conditions was most likely due to compensating the higher metabolic demand under these conditions, while feeding has also been shown to increase the buffering capacity of the coelomic fluid in sea urchins (Collard et al., 2013). This apparent link between feeding and metabolic function in sea urchins has also been reported in response to elevated temperatures in *Lytechinus semituberculatus* (Carr and Bruno, 2013), although another study reported that while the sea urchin experienced an increase in metabolic demand with OA, their feeding rates did not change (Carey et al., 2016). These differential metabolic and feeding responses to OA and/or temperature indicate species-specific responses, most likely related to differences in the capacity to increase consumption rates and in species' buffering capacity of the coelomic fluid (Collard et al., 2013). This is also supported by the wide range of responses in other parameters (i.e., calcification, growth, survival, etc.) (Shirayama and Thornton, 2005; Ries et al., 2009; see Dupont et al., 2010).

Photosynthesis and respiration rates of *J. rubens* were unaffected by treatments (Fig. 2a). Algal photosynthetic rates have shown to be highly variable to warming and acidification (Ji et al., 2016). For example, though mild warming usually increases photosynthetic rates in algae (Ji et al., 2016), some studies have found no effect of increased temperature on photosynthesis for both fleshy algae (Gutow et al., 2016) and calcareous algae (Martin et al., 2013), while others reported a decrease in photosynthesis in coralline algae (e.g., Tait, 2014; Kram et al., 2016). As mentioned above, the studied coralline algae inhabit intertidal rocky shores and are therefore most likely acclimated to a wide range in temperature.

Jania rubens did, however, suffer reduced $CaCO_3$ content in high CO_2 treatments, regardless of temperature (Fig. 2b). OA has been found to reduce $CaCO_3$ content in other studies of calcified algae (Johnson and Carpenter, 2012; Campbell et al., 2014), which may be due to either increased metabolic demand for calcification, or actual dissolution

of $CaCO_3$ structures (Kroeker et al., 2013a). $CaCO_3$ is incorporated into the cell walls of coralline red algae, and together with chemical defenses like phenolic compounds, serves as a deterrent to herbivory (Hay and Fenical, 1988). In contrast to the $CaCO_3$ content of the algal thallus, phenolic compounds were unaffected by OA, and did not show any response to the different temperature treatments (see Fig. 2c). Previous studies of phenolic compounds in macroalgae under climate change conditions have found conflicting results, including decreases (Yildiz and Dere, 2015), increases (Celis-Plá et al., 2015, 2017; Hargrave et al., 2016; Gouvêa et al., 2017), and no changes (Endo et al., 2013, 2015; Campbell et al., 2014; Hargrave et al., 2016; Vizzini et al., 2017). Thus, it appears changes in phenolic compounds caused by simulated climate change conditions may be species-specific, as found, for example, in the differential phenolic response to increasing temperature in two *Laminaria* species (Hargrave et al., 2016).

The effects of elevated temperature and/or OA on algal $CaCO_3$ content have been shown to affect herbivory rates indirectly. Two species of sea urchins, *Lytechinus variegatus* and *Diadema antillarum*, both increased feeding rates on the calcified green alga *Halimeda optunia* under acidified conditions; this was correlated with reduced algal $CaCO_3$ content, while there were no changes in the phenolic content (Campbell et al., 2014). Similarly, the crustose coralline alga *Porolithon onkodes* suffered reduced calcification rates under warming and acidification, and the sea urchin *Echinothrix diadema* increased consumption of algae in these treatments (Johnson and Carpenter, 2012). These studies lend support to the hypothesis that decreased $CaCO_3$ content increases the palatability of algae (i.e., Hay and Fenical, 1988). However, in the aforementioned cases the sea urchins were not exposed to the same treatment conditions as the algae, and therefore did not exhibit any changes in metabolic rates, as observed in the present study. Here, despite reduced $CaCO_3$ content in the acidified treatments, sea urchins only increased feeding rates in the low temperature, high CO_2 treatment (see Fig. 3b), suggesting that feeding rates by the sea urchin were driven by increased metabolism rather than changes to algal palatability (i.e., Sparks, 2017). This increase in feeding rate might have been amplified by the lower sugar content of the algae under this condition. Lower sugar content could reflect a less valuable food source for *E. lucunter* (You et al., 2014). This potential interplay between lower available energy provided by the algae, coupled with higher energy demand in the sea urchin, may result in a mismatch in algal production and herbivore consumption (Gutow et al., 2016). The diet of *E. lucunter* has been shown to depend on algal availability, rather than preferences (Mendes and Tavares, 2006). Thus, a reduced coralline algal abundance due to increased feeding would most likely increase their consumption of other, non-calcareous algae. This, however, might indirectly influence sea urchin susceptibility to OA, as it has been shown that under OA conditions coralline-algae-fed sea urchins exhibited stronger tests and jaws compared to ones fed with other algae, suggesting that the uptake of $CaCO_3$ from the diet might be fundamental for maintaining urchin defense against predation (Asnaghi et al., 2013).

5. Conclusion

Our study suggests that acidification will drive increased metabolism and feeding rates of the herbivore *E. lucunter*. This together with the complex interactive effects of suddenly increased temperature and OA on algal chemical composition may lead to changes in algal community composition. However, in order to predict larger community-level responses, further studies focusing on more species and their interactions are needed, as many effects of environmental change are taxon-specific and may scale differently with different species. For example, feeding rates of an amphipod scaled faster with increasing temperature than growth of a brown algal food source (Gutow et al., 2016), but turf algal primary production scaled faster with increasing temperature than consumption by a herbivorous gastropod (Mertens et al., 2015).

At this point, we want to stress that, while providing important information, the results of our experiment can only be interpreted in terms of sudden short-term environmental changes, such as those related to marine heat waves and rapid coastal acidification. Longer-term studies will be required in order to predict species' individual responses and interactions with other species, as well as their acclimation and adaptation capacity to gradual increases in seawater temperature and CO_2, which will shape near-future marine ecosystems.

Declarations of interest

None.

Author contribution

W.A.R., N.S., N.S., and P.A.H. contributed to designing the study, collecting and analyzing the data, and writing the paper. V.F.C. assisted with constructing and maintaining the mesocosm system and collecting data. A.C.L.H. designed and constructed the mesocosm system.

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